Immunity
Previews

Forewarned Is Forearmed

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Memory killer T cells contribute to control of secondary viral infection by exhibiting rapid effector function upon reinfection. In this issue of *Immunity*, Kohlmeier et al. (2010) demonstrate that type I interferon is key for rapid upregulation of effector function within circulating memory T cells, ensuring efficient control of infection.

Activated $CD8^+$ Cytotoxic T lymphocytes (CTLs) play a key role in the acute control of most virus infections. As the name suggests, CTLs are able to recognize and kill virally infected cells, thereby removing any potential reservoir for viral replication. Activated CTLs have cytoplasmic granules that contain a spectrum of cytotoxic proteins, including the pore-forming protein, perforin (pfp), and an array of serine proteases called granzymes (grz, granule enzymes). CTL mediate killing by depositing the contents of the cytoplasmic granules onto the target cell, whereupon perforin and granzymes work synergistically to initiate programmed cell death. Granzymes (grz) B and A are the most abundant granzymes within the cytolytic granules, and their expression is considered to be a signature of activated $CD8^+$ T cells (Smyth et al., 2001).

Upon infection, naive, virus-specific $CD8^+$ T cells are activated and undergo a program of proliferation and differentiation whereby they increase in number and start to express signature cytolytic molecules grzB and pfp (Jenkins et al., 2008). The acquisition of effector gene expression is tightly linked to antigen-dependent proliferation, and as such, it takes naive $CD8^+$ T cells about 2 to 3 days to express signature cytolytic proteins (Jenkins et al., 2008). Upon resolution of the infection, activated CTL numbers contract, leaving a pool of long-lived memory T cells. A hallmark of adaptive T cell immunity, memory CTLs provide enhanced protection from the ravages of a second infection. Key to this protective capacity, and in contrast to naive CD8+ T cells, is the ability of memory CTLs to display immediate effector function upon recognition of specific antigen. This is particularly true of memory T cells found to reside in peripheral tissues long after the infection has cleared (Gebhardt et al., 2009; Masopust et al., 2001). In contrast, studies have demonstrated that circulating memory CTL exhibit little or no expression of cytolytic machinery and display diminished in vivo and/or ex vivo cytolytic activity (Jenkins et al., 2007; Masopust et al., 2001). As such, circulating memory T cells can be considered quiescent, yet these same memory CTLs are recruited quickly to the site of infection and contribute to early control of virus infection (Kohlmeier et al., 2008). Thus, a key question in the field is how do circulating memory CTLs act so quickly to provide protection from reinfection when it is clear that these cells exhibit poor cytolytic capacity?

Kohmeier et al., (2010) have investigated how these circulating memory CTLs are able to respond with such rapidity to secondary viral challenge. Using non-crossreactive models of sendai and influenza respiratory virus infections in B6 mice, Kohlmeier et al. (2010) were able to assess the impact of non-antigen-specific (influenza virus-induced) inflammation on pre-existing Sendai-specific CTL memory populations. Strikingly, respiratory infection with an unrelated influenza A virus induced peak grzB expression within sendai virus-specific memory CTLs within 2 to 3 days after infection. Expression of grzB was not limited to memory CTLs found in the infected lung but was also observed in memory CTLs located at distant anatomical sites from the infection. This antigen-independent upregulation of grzB correlated with superior ex vivo antigen-specific cytotoxicity compared to memory CTLs taken from mock challenged mice.

The rapid and systemic induction of grzB expression within circulating memory CTLs pointed to a key role for nonspecific inflammation. It has long been appreciated that such inflammation, particularly type I interferon (IFN) signaling, plays a key role in ensuring effective primary T cell responses to infection. Although type I IFN signals also appear to be important for establishment of robust memory populations (Kolumam et al., 2005), little is known about their role in promoting recall T cell responses. In fact, previous work has suggested that induction of type I IFNs after heterologous infection results in attrition of pre-existing memory T cell numbers (McNally et al., 2001). This has been proposed as a mechanism for accommodating newly generated memory T cell populations in the memory T cell compartment. Through a series of bone marrow chimera experiments, Kohlmeier et al. demonstrate that the nonspecific induction of granzyme B expression and enhanced cytolytic ability were dependent on STAT 1 transcription factor signaling and, specifically, signaling through the type I IFN receptor. That type I IFN signaling was both necessary and sufficient to upregulate granzyme B was evidenced by the enhanced expression of granzyme B after addition of IFN-α or IFN-β to memory CTLs from mice or to cultures of human peripheral blood mononuclear cells. Thus, the phenomena of upregulated granzyme B expression along with enhanced cytolytic ability as a consequence of type I interferon signaling are convincingly established in this study (Figure 1).

Critically, however, the consequences of the burgeoning cytolytic potential implied by the earlier experiments, and the real physiological relevance of these phenomena, are only revealed when the established memory population was primed and challenged with antigenically related viruses. This enabled the consequence of nonspecific effects of the inflammatory environment on antigen-specific

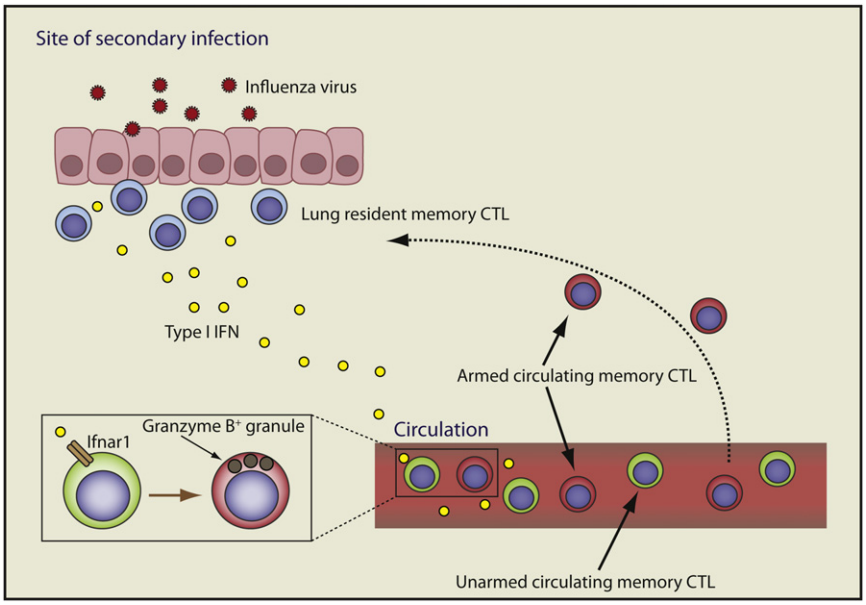

Figure 1. Type I IFN Signaling Induces Immediate Cytolytic Capacity in Circulating Memory CTL

Upon secondary influenza infection, systemic inflammatory signals are induced that include production of type I interferons (IFN). The lung-resident memory CTLs, while able to provide some control of infection, are not cytolytic. Circulating memory CTLs do not express granzyme B and are, hence, noncytolytic (unarmed). Signaling via systemic type I IFN induces antigen-independent granzyme B protein expression within circulating memory CTLs and imparts cytolytic function. These "armed" CTLs are then recruited to site of infection where they help limit viral replication.

CTL memory recall responses to be determined. In this elegant set of experiments, chimeric mice that had received bone marrow from either wild-type or *Ifnar1*$^{-/-}$ mice were primed and challenged with related influenza viruses. Despite the ability of all memory CTLs to recognize specific antigen, wild-type memory CTLs showed substantially elevated amounts of granzyme B after challenge compared to *Ifnar1*$^{-/-}$ cells. Furthermore, this difference corresponded to substantially lower viral titers 3 days after influenza challenge. As such, these data convincingly explain how memory CTLs recruited to the site of infection are able to respond with such rapidity after secondary infection.

Kohlmeier et al. demonstrate that the rapid cytolytic response that is a consequence of priming by type I IFN signaling appears limited to cells entering the affected tissue from the circulation and not those resident in the lungs. Their data clearly show that tissue resident memory cells (as defined by low expression of CD11a) exhibit only a modest increase in granzyme B expression and remain poorly cytolytic compared to memory cells entering the lungs after challenge. These data appear contradictory to earlier studies by this group (Hogan et al., 2001) and others (Gebhardt et al., 2009; Masopust et al., 2001) that have demonstrated the protective capacity of tissue resident memory CTLs. It is also is in direct contrast to earlier studies that have demonstrated tissue-resident virus-specific memory CTL established in models of *Listeria monocytogenes*, vesicular stomatits virus, or herpes simplex virus do, in fact, exhibit enhanced cytolytic activity compared to circulating memory T cells (Gebhardt et al., 2009; Masopust et al., 2001). The reasons for these differences are unclear and suggest that the route of infection or the type of pathogen may influence the expression of type I IFN receptors by the CTLs. This needs to be assessed in these models of nonrespiratory infection. Moreover, it is possible the lung microenvironment may actively suppress cytolytic activity by tissue-resident memory CTLs. It makes sense to ensure that such a potent effector mechanism is tightly controlled in such a sensitive tissue. Thus, this study indicates that the protection afforded by tissue-resident memory CTLs is mediated by noncytolytic functions; however, the precise mechanism remains to be determined. Similarly, how these two populations of memory cells, circulating and resident, act in concert to promote viral clearance remains to be elucidated. Finally, understanding the events that render memory T cells responsive to type I IFN signaling and, therefore, more rapid responses will be key for improving strategies designed to promote effective cellular immunity.

In summary, this study provides an intriguing solution to the quandary of how resting memory cells react so quickly after antigen re-encounter. Unlike quiescent naive cells, whose acquisition of cytolytic capacity is only initiated upon antigen recognition, circulating memory cells are alerted to the presence of infection, allowing them to arm themselves prior to antigen encounter. This prime example of collaboration between innate and adaptive immunity thus epitomizes the adage "forewarned is forearmed."

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